

In the claims

The following amendments are made with respect to the claims in the International application PCT/EP2004/002216.

This listing of claims will replace all prior versions and listings of claims in this application.

1 (original). A conditionally inducible site-directed mutant cell, comprising

- a) a mutated allele of a gene; wherein said allele comprises a mutation that was introduced by using a suitable mutagenesis technique,
  - b) a rescue allele of said mutated gene that can be conditionally inactivated,
- wherein said mutation in said mutated allele of said gene interferes with survival and/or causes an adverse phenotype.

2 (currently amended). The conditionally inducible site-directed mutant cell according to claim 1, wherein said mutated allele of said gene comprises a mutation at the exon or sub-exon level, wherein said mutation is selected from the group consisting of ~~such as a~~ deletions, point mutations, insertions, and ~~inversions, and the like.~~

3 (currently amended). The conditionally inducible site-directed mutant cell according to claim 1[[ or 2]], wherein said rescue allele and/or its transcription product(s) comprises recombination target sites, ~~e.g. lox or FRT sites,~~ sites for the attachment of antisense oligonucleotides, ~~e.g. DNA, PNA and/or RNA oligonucleotides,~~ sites for ribozyme activities, and/or sites that interfere with specific siRNA for expression.

4 (currently amended). The conditionally inducible site-directed mutant cell according to claim 1[[ or 2]], wherein said rescue allele comprises a conditionally inducible genetic construct which either directly or via its expression product inhibits the function of any non-mutated copy of said mutated allele.

5 (currently amended). The conditionally inducible site-directed mutant cell according to ~~any of claims 1 to 4~~ claim 1, containing multiple mutated alleles of genes and/or a multiply mutated allele of a gene together with their suitable rescue allele(s).

6 (currently amended). The conditionally inducible site-directed mutant cell according to ~~any of claims 1 to 5~~ claim 1, wherein said allele encodes ~~[[for]]~~ titin.

7 (currently amended). The conditionally inducible site-directed mutant cell according to ~~any of claims 1 to 6~~ claim 1, wherein said interference with survival and/or adverse phenotype is selected from temporal and/or local phenotypes, ~~such as cell cycle specific, cell type specific, tissue specific, protein expression specific, tissue development specific, organ specific, organ development specific and/or embryonic lethal phenotypes.~~

8 (currently amended). The conditionally inducible site-directed mutant cell according to ~~any of claims 1 to 7~~ claim 1, which is selected from a prokaryotic cell, a eukaryotic cell, a diploid cell, a plant cell, a mammalian cell, a nematode cell, a fish cell, an insect cell, and; ~~in particular,~~ a non-human stem-cell.

9 (currently amended). A conditionally inducible site-directed mutant cell culture, tissue, organ, ~~[[or]]~~ non-human embryo, or non-human organism comprising a ~~cell according to any of claims 1 to 8~~ conditionally inducible site-directed mutant cell, comprising

a) a mutated allele of a gene; wherein said allele comprises a mutation that was introduced by using a suitable mutagenesis technique,

b) a rescue allele of said mutated gene that can be conditionally inactivated, wherein said mutation in said mutated allele of said gene interferes with survival and/or causes an adverse phenotype.

10 (cancelled).

11 (currently amended). The conditionally inducible site-directed mutant non-human organism according to claim ~~[[10]]~~ 9, containing multiple mutated alleles of genes and/or a multiply mutated allele of a gene together with their suitable rescue allele(s).

12 (currently amended). The conditionally inducible site-directed mutant non-human organism according to claim ~~9~~ 9 ~~[[ or 10]]~~, wherein said interference with survival and/or adverse phenotype is selected from temporal and/or local phenotypes, ~~such as cell cycle specific, cell type specific, tissue specific, tissue development specific, protein expression specific, organ specific, organ development specific and/or embryonic lethal phenotypes.~~

13 (original). A method for producing an inducible site-directed mutant cell capable of conditional gene rescue, comprising

- a) introducing in a target cell a mutated allele of a gene to be mutated by using a suitable mutagenesis technique,
  - b) introducing in said target cell a rescue allele of said gene that can be conditionally inactivated, and
  - c) optionally, cultivating said target cell under conditions that allow for a selection of cells that contain both the mutated allele and the rescue allele of said gene,
- wherein said mutation in said mutated allele of said gene interferes with survival and/or causes an adverse phenotype.

14 (currently amended). The method according to claim 13, wherein said suitable mutagenesis technique comprises introducing a mutation at the exon or sub-exon level, ~~such as a deletions, point mutations, insertions, inversions, and the like, preferably by using a suitable mutagenesis technique employing a vector system, irradiation, random integration of foreign DNA, site specific recombination, homologous recombination, and/or chemical mutagenesis.~~

15 (currently amended). The method according to claim 13[[ or 14]], wherein introducing said rescue allele comprises transfection or infection of the cell with a rescue allele genetic construct comprising recombination target sites, ~~e.g. lox or FRT sites,~~ sites for the attachment of antisense oligonucleotides, ~~e.g. DNA, PNA and/or RNA oligonucleotides,~~ sites for ribozyme activities, and/or sites that interfere with specific siRNA for expression.

16 (currently amended). The method according to claim 13[[ or 14]], wherein introducing said rescue allele comprises transfer of a conditionally inducible genetic construct into the cell, which either directly or via its expression product inhibits the function of any non-mutated copy of said mutated allele.

17 (currently amended). The method according to ~~any of claims 13 to 16~~ claim 13, wherein a tissue specific rescue allele and/or mutated allele is introduced.

18 (currently amended). The method according to ~~any of claims 13 to 17~~ claim 13, wherein said allele encodes [[for]] titin.

19 (currently amended). The method according to ~~any of claims 13 to 18~~ claim 13, wherein said cell is selected from a prokaryotic cell, a eukaryotic cell, a diploid cell, a plant cell, a mammalian cell, a fish cell, a nematode cell, an insect cell, and, ~~in particular~~, a non-human stem-cell.

20 (currently amended). The method according to ~~any of claims 13 to 19~~ claim 13, comprising the introduction of multiple mutated alleles of genes and/or a multiply mutated allele of a gene together with their suitable rescue allele(s).

21 (currently amended). The method according to ~~any of claims 13 to 20~~ claim 13, wherein said interference with survival and/or adverse phenotype is selected from temporal and/or local phenotypes, ~~such as cell cycle specific, cell type specific, tissue specific, tissue development specific, organ specific, organ development specific and/or embryonic lethal phenotypes.~~

22 (currently amended). The method according to ~~any of claims 13 to 20~~ claim 13, further comprising

- d) conditionally inactivating said rescue allele of said gene to be mutated by using a suitable inactivation technique.

23 (currently amended). The method according to claim 22, wherein conditionally inactivating said rescue allele of said gene to be mutated by using a suitable inactivation technique comprises a technique selected from site directed recombination, ~~such as cre/lox or Flp/FRT inactivation~~, antisense inactivation using oligonucleotides, ~~e.g. DNA, PNA and/or RNA oligonucleotides~~, RNA-interference, ~~such as ribozyme activity inactivation~~, siRNA expression-inactivation, inactivation of the gene product (protein) and/or its activity and/or inducible inactivation of the non-mutated allele, ~~such as through antibodies~~, inactivation of the activity of a fusion protein or induced proteolysis.

24 (currently amended). The method according to ~~any of claims 13 to 23~~ claim 13, wherein said method is performed in vivo or in vitro.

25 (currently amended). The method according to ~~any of claims 13 to 24~~ claim 13, wherein said cell is present in a tissue, organ, non-human embryo or non-human organism; ~~in particular a mammal, rodent, nematode, fish, plant, or insect.~~

26 (currently amended). A method for the production of an inducible site-directed non-human mutant-organism comprising a cell capable of conditional gene rescue, comprising

a) generating an inducible site-directed mutant cell by a method comprising

i) introducing in a target cell a mutated allele of a gene to be mutated by using a suitable mutagenesis technique,

ii) introducing in said target cell a rescue allele of said gene that can be conditionally inactivated, and

iii) optionally, cultivating said target cell under conditions that allow for a selection of cells that contain both the mutated allele and the rescue allele of said gene,

wherein said mutation in said mutated allele of said gene interferes with survival and/or causes an adverse phenotype; and

~~according to the method according to any of claims 13 to 24~~

b) generating a non-human mutant organism comprising said mutant cell.

27 (currently amended). An inducible site-directed non-human mutant-organism, produced according to ~~claim 26, in particular a mammal, nematode, rodent, fish, plant, or insect a~~ method comprising

a) generating an inducible site-directed mutant cell by a method comprising

i) introducing in a target cell a mutated allele of a gene to be mutated by using a suitable mutagenesis technique,

ii) introducing in said target cell a rescue allele of said gene that can be conditionally inactivated, and

iii) optionally, cultivating said target cell under conditions that allow for a selection of cells that contain both the mutated allele and the rescue allele of said gene,

wherein said mutation in said mutated allele of said gene interferes with survival and/or causes an adverse phenotype; and

b) generating a non-human mutant organism comprising said mutant cell.

28 (new). The method, according to claim 3, wherein said rescue allele and/or its transcription product(s) comprises lox or FRT sites.

29 (new). The method, according to claim 7, wherein said temporal and/or local phenotype is selected from the group consisting of cell cycle-specific, cell-type specific, tissue-specific, protein-expression specific, tissue-development specific, organ-specific, organ-development-specific and embryonic lethal phenotypes.

30 (new). The mutant non-human organism according to claim 12 wherein said temporal and/or local phenotype is selected from the group consisting of cell cycle-specific, cell-type specific, tissue-specific, protein-expression specific, tissue-development specific, organ-specific, organ-development-specific and embryonic lethal phenotypes.

31 (new). The method, according to claim 14, wherein said suitable mutagenesis technique employs a vector system, irradiation, random integration of foreign DNA, site specific recombination, homologous recombination, or chemical mutagenesis.

32 (new). The method, according to claim 21, wherein said temporal and/or local phenotype is selected from the group consisting of cell cycle-specific, cell-type specific, tissue-specific, protein-expression specific, tissue-development specific, organ-specific, organ-development-specific and embryonic lethal phenotypes.

33 (new). The method, according to claim 23, wherein said inactivation technique is selected from the group consisting of cre/lox or Flp/FRT inactivation; ribozyme activity inactivation; and inactivation of the non-mutated allele using an antibody.

34 (new) The method, according to claim 25, wherein said non-human organism is a mammal, rodent, nematode, fish, plant, or insect.